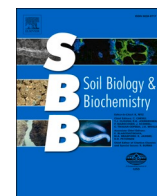


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## Sticky dead microbes: Rapid abiotic retention of microbial necromass in soil

Kate M. Buckeridge<sup>a,\*</sup>, Alfio Fabio La Rosa<sup>a,2</sup>, Kelly E. Mason<sup>b</sup>, Jeanette Whitaker<sup>b</sup>,  
Niall P. McNamara<sup>b</sup>, Helen K. Grant<sup>b</sup>, Nick J. Ostle<sup>a</sup>

<sup>a</sup> Lancaster Environment Centre, University of Lancaster, Lancaster, UK<sup>b</sup> UK Centre for Ecology & Hydrology, Lancaster, UK

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## ABSTRACT

Microbial necromass dominates soil organic matter. Recent research on necromass and soil carbon storage has focused on necromass production and stabilization mechanisms but not on the mechanisms of necromass retention. We present evidence from soil incubations with stable-isotope labeled necromass that abiotic adsorption may be more important than biotic immobilization for short-term necromass retention. We demonstrate that necromass adsorbs not only to mineral surfaces, but may also interact with other necromass. Furthermore, necromass cell chemistry alters necromass-necromass interaction, with more bacterial tracer retained when there is yeast necromass present. These findings suggest that the adsorption and abiotic interaction of microbial necromass and its functional properties, beyond chemical stability, deserve further investigation in the context of soil carbon sequestration.

## 1. Main text

Soil organic matter (SOM) is a key indicator for healthy soil and sustainable agriculture (Paustian et al., 2016). Research into SOM stability has traditionally focused on the quality and quantity of plant inputs to soil (Lehmann and Kleber, 2015), however, recent research reveals that SOM is dominated by dead microbial products and residues (hereafter, 'necromass' (Kallenbach et al., 2015; Liang et al., 2019, 2011)). The persistence of necromass in soil may be promoted via necromass uptake and immobilization into microbial biomass, but retention ultimately relies on microaggregate formation via adsorption to soil mineral surfaces. In actuality, both biotic (microbial immobilization) and abiotic retention (adsorption and molecular interaction) may co-occur but the relative importance of these short-term processes has not been assessed (Fig. 1).

Necromass has been visualized on mineral surfaces (Kleber et al., 2011), supporting the paradigm that accumulation of stable SOM is dominated by organic-mineral adsorption, and is limited by mineral surface area (McNally et al., 2017). However, necromass is not detected as a smooth coverage on mineral surfaces, but clumpy (Dignac et al., 2017; Vogel et al., 2014), suggesting that SOM stabilization may also involve organic-organic interactions, or necromass adhering to other

necromass and organic matter for example by ionic interactions, hydrogen bridges, van der Waals forces, and (partial) entrapment (Schweizer et al., 2018; Vogel et al., 2014) (Fig. 1). Understanding the relative importance of these two abiotic adsorption processes will be critical for predicting upper limits of SOM persistence.

The chemistry of microbial necromass has been assumed unimportant as a regulator of SOM storage, because its chemical composition is more similar than diverse plant inputs (Liang et al., 2017). Previous research on necromass chemistry and persistence has focused on its stability and emphasized chitin retention (Fernandez et al., 2016; Schreiner et al., 2014). However, cell chemistry can alter rates of cell-cell adhesion (Dufrène, 2015) and organic-mineral adsorption rates (Meissner et al., 2015), particularly for N-rich necromass (Kopittke et al., 2017). Gram-positive bacterial envelopes have a thick cross-linked peptidoglycan layer outside the lipid membrane, whereas a Gram-negative cell envelope has an inner and outer lipid membrane enclosing a thinner peptidoglycan layer in the periplasm. Fungal cell walls are highly heterogeneous; yeast cell walls, for example, are composed of layered mannan,  $\beta$ -glucans and chitin outside a lipid membrane. Therefore, cell-membrane functional groups with a high N-content, such as peptidoglycan-rich Gram-positive bacteria, may be favored for organic-organic interaction, relative to Gram-negative

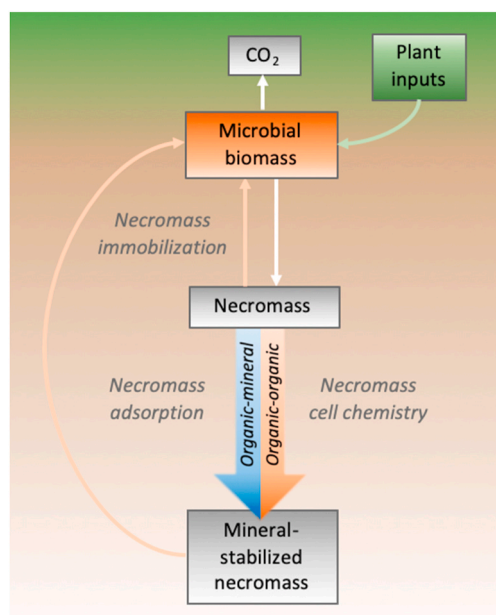
\* Corresponding author.

E-mail address: [kmbuckeridge@gmail.com](mailto:kmbuckeridge@gmail.com) (K.M. Buckeridge).<sup>1</sup> Present address: Global Academy of Agriculture and Food Security, The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK.<sup>2</sup> Present address: Department of Mathematics, University of Bonn, Bonn, DE.<https://doi.org/10.1016/j.soilbio.2020.107929>

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**Fig. 1.** Schematic diagram of microbial necromass fate in mineral soils. In this diagram, necromass is presented as a substrate for microbes, similar to plant inputs (litter or exudates). Microbes can acquire unassociated or mineral-associated necromass for substrate. Immobilization includes recycling of necromass by microbes into new biomass, and eventually necromass, with potential for some loss as CO<sub>2</sub>. Stabilization is assumed to be through adsorption to mineral surfaces ('organic-mineral'), especially on fine silts and clays. In this study we hypothesize that this process is not limited to mineral surface availability ('organic-mineral'), but that necromass also adheres to necromass ('organic-organic'), promoting retention, and that this organic-organic process may be influenced by necromass cell chemistry.

bacteria or fungi. Given the potential for broad shifts in microbial community composition as a result of global change, land use change or even seasonality (Buckeridge et al., 2013; Ramirez et al., 2012), necromass cell chemistry may influence SOM stabilization at ecosystem scales.

We investigated the importance of biotic and abiotic necromass retention in grassland soil and the influence of necromass chemistry on this retention in short-term laboratory incubations. We hypothesized: H1. both biotic and abiotic necromass retention occur, and that biotic retention is more important; H2. more necromass is retained in soil with higher background concentrations of necromass (implying organic-organic adhesion); and H3. abiotic retention would be higher for Gram-positive bacterial membranes (implying cell chemistry is important). We tested these hypotheses with short-term (3-d) laboratory incubations of clay-loam agricultural pasture soil; the duration was chosen to limit biotic processing of rapid abiotic interactions. Detailed methods are available in supplementary methods. Soil was assigned to live and sterile treatments with enhanced background necromass (~5.5 mg C and ~1.5 mg N g<sup>-1</sup> soil fwt) from three soil microbial functional groups (Gram-negative (*Escherichia coli*) and Gram-positive bacteria (*Micrococcus luteus*) and yeast (*Saccharomyces cerevisiae*)), and incubated with a no-addition control. We also added a small amount of isotopic tracer necromass consisting of <sup>13</sup>C<sup>15</sup>N labeled (10 atm%) *E.coli* necromass (0.050 mg C and 0.016 mg N g<sup>-1</sup> soil fwt) to all treatments; the dual label was used to aid assessment of biotic vs. abiotic retention. Head-space CO<sub>2</sub> and N<sub>2</sub>O samples were collected throughout the 3-day incubation. At the end of the incubation, we used the chloroform-direct-extraction method (Fierer et al., 2003) to estimate microbial biomass immobilization of the tracer (measured in all, detected only in live soils), and the balance between immobilization and loss as CO<sub>2</sub> was used to calculate carbon use efficiency of the live microbes. The C, N, δ<sup>13</sup>C and

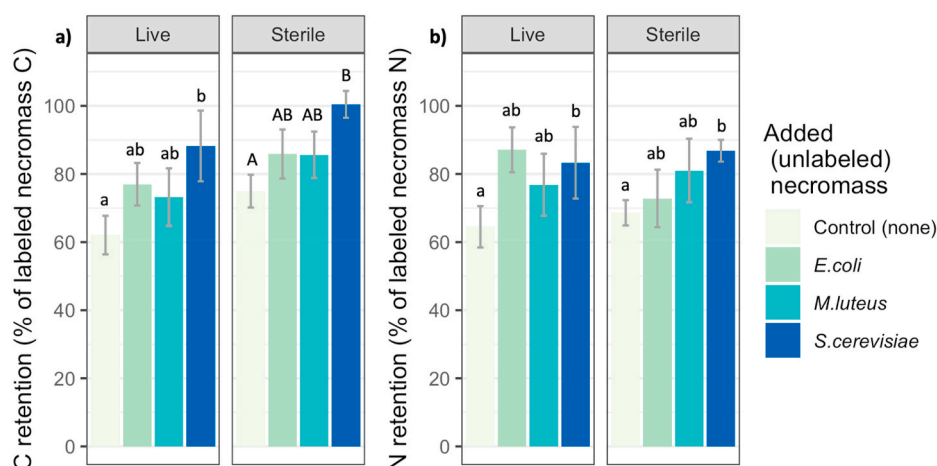
δ<sup>15</sup>N of the post-extraction soils (i.e. removing any free or loosely-bound C or N) was used to assess total retention (assumed biotic + abiotic in the live soils and abiotic only in the sterile soils), and mixed models were used to assess the statistical significance ( $\alpha = 0.05$ ) of our results.

Retention of C from the tracer necromass was lower in live vs. sterile soils ( $P = 0.006$ ), rejecting H1 and indicating that short-term retention of necromass C was dominated (>70%) by abiotic processes (Fig. 2a). Our results, however, cannot confirm that biotic processes are unimportant for persistent SOM-C accrual. Our controlled environment incubation may have overestimated the importance of abiotic processes: in a more dynamic, natural system with active plant-microbial interactions, live microbial immobilization of necromass-C and plant and microbial uptake/immobilization of necromass-N is potentially critical to retention. Furthermore, our lab-grown, single-culture additions only approximate the retention of chemically and taxonomically-complex native necromass. Nonetheless, our results illustrate an important short-term effect of abiotic C retention in soil.

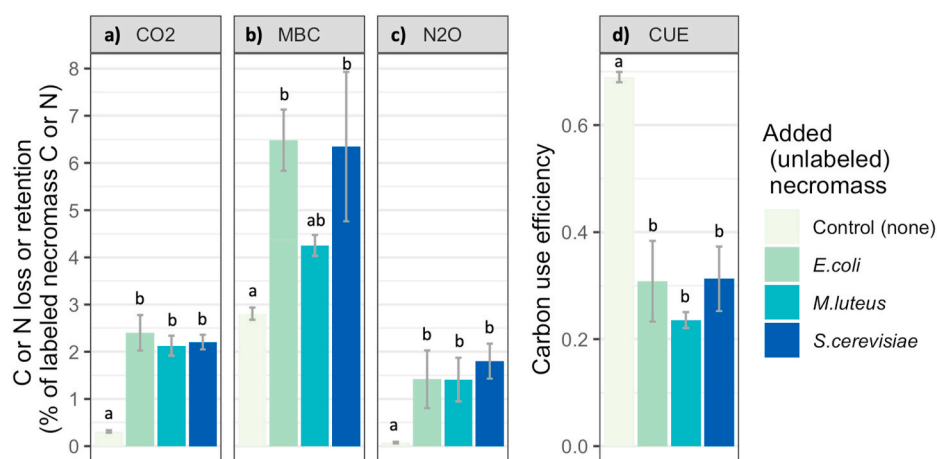
The lower retention of <sup>13</sup>C in live vs. sterile soils was not predicted and we suspected this was either a side-effect of sterilization, or CO<sub>2</sub> loss from microbial activity. We discounted the sterilization effect, because we did not see a parallel lower retention of necromass-<sup>15</sup>N in live compared to dead soils (Fig. 2b). This, combined with no change in C:N ratio of tracer retention in sterile soils and a drop in C:N ratio of retention in live soils ( $P < 0.001$ , data not shown) equates with live microbial processing of C, associated with immobilization/loss of 4–10% of the total C added, as extractable biomass or CO<sub>2</sub> (Fig. 3a and b). Losses of N<sub>2</sub>O from the labeled necromass were comparable to CO<sub>2</sub> (<2%, Fig. 3c) but we presume that N uptake to microbial biomass (not measured) would be lower than C, reflecting the lower N demand for growth in these N-rich pasture soils. C-starvation during incubation may have resulted in use of necromass-C for maintenance, reflected in a low substrate use efficiency (CUE) (Fig. 3d).

Higher C and N was retained in live and sterile soils with enhanced background necromass compared to controls (~10–40% higher, Fig. 2a and b), supporting the hypothesis (H2) that necromass may be adhering to necromass. Organic-organic adhesion has been suggested previously based on isotopic and visual evidence in laboratory and long-term field studies (Schweizer et al., 2018). We did not observe higher C and N retention in soils with enhanced peptidoglycan (*M. luteus*) necromass, rejecting H3, that abiotic retention would be higher for Gram-positive bacterial membranes. However, we provide novel evidence that the retention of C and N from the necromass tracer was higher in live and dead soils augmented with *S. cerevisiae* necromass (C:  $P < 0.001$ ; N:  $P = 0.03$ ). This higher microbial C and N retention in *S. cerevisiae*-amended soils does not appear to be a biotic process, because the CUE of the tracer necromass in live soils did not differ between background necromass types (Fig. 3d) (despite all enhanced background necromass treatments being lower than the no-addition control, which was presumably a response to higher substrate concentration relative to the control (Geyer et al., 2019)). Instead, this enhanced retention of the tracer necromass in the presence of *S. cerevisiae* necromass may be indicative of faster or stronger interaction between the complex morphogenesis of the Gram-negative outer cell membrane and yeast cell walls, such as occur in live microbial communities (Dufrène, 2015). Further compound-specific research is required to understand if the properties of yeast necromass extend to other fungal necromass generally and to specific bacterial membrane and fungal cell wall compounds. Regardless, this result indicates that cell chemistry contributes to an adhesion mechanism that promotes necromass stability in soil.

We conclude that abiotic processes are important for short-term retention of necromass-C and N in soils and require greater emphasis in studies investigating SOM stability. Our results indicate that organic-organic interactions promote retention of C and N and contribute novel evidence that this mechanism is regulated by cell chemistry. If this short-term abiotic retention occurs *in situ* and persists, then microbial community structure and possibly the fungal:bacterial ratio, may influence C



**Fig. 2.** The retention of necromass ( $^{13}\text{C}^{15}\text{N}$ -*E. coli*) carbon and nitrogen in live and sterile soil with different background necromass. Data presented as the mean ( $\pm$ standard error,  $n = 5$ ) % retention of applied  $^{13}\text{C}^{15}\text{N}$  necromass as (a) carbon (C) or (b) nitrogen (N) after 3 days incubation in live (microbial immobilization and adsorption) and sterile (adsorption only) agricultural pasture soils. Tracer  $^{13}\text{C}^{15}\text{N}$ -labeled *E. coli* necromass was added (0.050 mg C and 0.016 mg N  $\text{g}^{-1}$  soil fwt) in combination with different background (i.e. unlabeled) necromass (control, *E. coli*, *M. luteus* or *S. cerevisiae* ( $\sim 5.5$  mg C and  $\sim 1.5$  mg N  $\text{g}^{-1}$  soil fwt)). Sterile soils were either autoclaved or gamma-irradiated (results pooled) and thus presumably have a slightly higher level of background necromass C and N from the recently killed natural soil microbial community ( $\sim 0.38$  mg C and 0.030 mg N  $\text{g}^{-1}$  soil fwt). 'Retention' is calculated from the amount of label remaining in the water-extracted soil residue at the end of a short-term (3 d) incubation. Upper and lower-case letters within each plot indicate a significant difference between live and sterile treatments, and different letters indicate significant difference between necromass treatments ( $\alpha = 0.05$ ).



**Fig. 3.** Tracing the gaseous loss, biotic immobilization and carbon use efficiency of necromass tracer ( $^{13}\text{C}^{15}\text{N}$ -*E. coli*) in live soils with different background necromass. Data presented as the mean ( $\pm$ standard error,  $n = 5$ ) % of applied  $^{13}\text{C}^{15}\text{N}$  necromass in (a)  $\text{CO}_2$ , (b) microbial biomass ('MBC'), and (c)  $\text{N}_2\text{O}$ , and (d) the carbon use efficiency ('CUE') of necromass mineralization after 3 days incubation. Tracer  $^{13}\text{C}^{15}\text{N}$ -labeled *E. coli* necromass was added (0.050 mg C and 0.016 mg N  $\text{g}^{-1}$  soil fwt) in combination with different background (i.e. unlabeled) necromass (control, *E. coli*, *M. luteus* or *S. cerevisiae* ( $\sim 5.5$  mg C and  $\sim 1.5$  mg N  $\text{g}^{-1}$  soil fwt)). Different letters within each plot indicate significant difference between necromass treatments ( $\alpha = 0.05$ ).

and N stabilization through variations in community cell chemistry. Field additions of isotopically-labeled necromass from different taxa, in different soils), would be valuable for investigating the long-term importance of these mechanisms. These findings suggest that abiotic adsorption and interaction of microbial necromass and its functional properties beyond chemical stability (i.e. cell molecular property, aggregations, and morphology), deserve further investigation in the context of soil carbon sequestration.

#### Data availability

The supporting data and R code for the statistics and figures in this paper can be found at: <https://doi.org/10.5281/zenodo.3957441>.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2020.107929>.

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